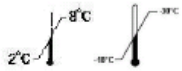
DATA SHEET

HPV High Risk Screen

Key to symbols used

REF	List Number		Store at +2-8/-20°C
IVD	For *in Vitro* Diagnostic Use		Caution!
LOT	Lot Number	VER	Version
	Expiration Date		Consult instructions for use
CONTROL −	Negative Control	CONTROL +	Positive Control
	Contains reagents		Manufacturer

NAME

HPV High Risk Screen

INTRODUCTION

Genital infection with HPV is one of the most common sexually transmitted diseases (STDs) of viral etiology worldwide (20% - 46% in different countries in sexually active young women).

Cervical cancer is the second most common cancer in women worldwide, and a compelling body of clinical, epidemiological, molecular, and experimental evidence has established the etiological relationship between some sexually transmitted HPV genotypes and cervical neoplasia throughout the world. Based on the frequency of detection of HPV genotypes from different grades of Cervical Intraepithelial Neoplasia (CIN Grades I – III), HPV genotypes are subdivided into High-risk HPV types (16, 18, 31 and 45), Intermediate-risk types (33, 35, 39, 51, 52, 56, 58, 59, and 68), and Low-risk types (6, 11, 42-44).

Several methods have been used to diagnose clinical or subclinical infection with HPVs including clinical observation, cytological screening by Pap smear, electron microscopy, immunocytochemistry, but these methods have some disadvantages such as non-standardization and subjectivity, insufficient sensitivity and low predictable value. The most perspective way of HPV diagnosis is a direct detection of DNA of the human papilloma virus of high carcinogenic risk by the polymerase chain reaction. While the value of the Pap smear in routine screening for cervical displasia is undisputed, it is now known that 99% of cases of cervical carcinoma are caused by infection with twelve genotypes of the human papilloma virus (HPV). Identification of these high-risk genotypes is very valuable in the management of cervical carcinoma, both as a prognostic indicator and as a secondary screening test where results of a Pap smear are inconclusive. Results from the combination of the Pap smear and the HPV DNA test can aid in determining the intervals for screening.

The PCR-based methods have been used successfully for the detection and typing of genital HPV genotypes in clinical specimens such as cervical swabs or scrapes, cervicovaginal lavages, frozen biopsies and formalin-fixed paraffin-embedded tissues.

INTENDED USE

HPV High Risk Screen is an *in vitro* nucleic acid amplification test for qualitative detection of *Human Papillomavirus* (16, 18, 31, 33, 35, 39, 45, 52, 53, 56, 58, 59, 66, 70*)* in the urogenital swabs and biopsies.

PRINCIPLE OF ASSAY

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HPV High Risk Screen Test is based on three major processes: sample preparation, multiplex amplification of DNA
using specific *HPV* primers and detection of the amplified products on agarose gel. PCR-mix-1 tube contains primers directed against regions of *HPV* A6, A7, A9 groups (HPV types 16, 18, 31, 33, 35, 39, 45, 52, 53, 56, 58,
59, 66, 70) and β-globine gene used as Internal Control. If the swab is not correctly prepared (high quantity of mucous or insufficient quantity of epitelial cells) the Internal Control will not be detected.

MATERIALS PROVIDED

- **PCR-mix-1**, 0,275 mL
- **2,5 x buffer**, 0,6 mL
- **TaqF Polymerase,** 0,03 mL
- **Mineral Oil**, 2,0 ml
- **Negative Control***, 1,2 ml;
- **DNA-buffer (C-)**, 0,5 mL
- **Internal Control** (β-globine gene), 0,1 mL
- **HPV C+ Positive Control** (types 31, 39, 56), 1 x 0,1 ml

Contains reagents for 55 samples.
*must be used in the isolation procedure as Negative Control of Extraction.

MATERIALS REQUIRED BUT NOT PROVIDED

- Thermalcycler
- Workstation
- Pipettors (capacity 0,5-10 µl; 5-40 µl) with aerosol barrier
- Tube racks

Reagents non provided

- DNA extraction kit (recommended nucleic acid extraction kit: DNA-Sorb-A (Sacace, REF K-1-1/A)
- Detection agarose kit

WARNINGS AND PRECAUTIONS

1. Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterward.
2. Do not pipette by mouth. Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work
areas.
3. Do not use a kit after its expiration date.
4. Dispose of all specimens and unused reagents in accordance with local regulations.
5. Biosafety Level 2 should be used for materials that contain or are suspected of containing infectious agents.
6. Clean and disinfect all spills of specimens or reagents using a disinfectant such as 0,5% sodium hypochlorite, or
other suitable disinfectant.
7. Avoid contact of specimens and reagents with the skin, eyes and mucous membranes. If these solutions come into contact, rinse immediately with water and seek medical advice immediately.
8. Material Safety Data Sheets (MSDS) are available on request.
9. This kit is designed for use with "DNA-Sorb" extraction kit. It is the user's responsibility if kits other than "DNA-Sorb" are used to perform this DNA extraction.
10. Use of this product should be limited to personnel trained in the techniques of amplification.
11. Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where you performed previous step.

STORAGE INSTRUCTIONS

HPV High Risk Screen must be stored at 2-8°C. Store **TaqF Polymerase** at -20°C. The kit can be shipped at 2-8°C for 3-4
days but should be stored at 2-8°C and -20°C immediately on receipt.

STABILITY

HPV High Risk Screen is stable up to the expiration date indicated on the kit label. The product will maintain performance through the control date printed on the label. Exposure to light, heat or humidity may affect the shelf
life of some of the kit components and should be avoided. Repeated thawing and freezing of these reagents should be avoided, as this may reduce the sensitivity. Components stored under conditions other than those stated on the labels

may not perform properly and may adversely affect the assay results.

SAMPLE COLLECTION, STORAGE AND TRANSPORT

HPV High Risk Screen can analyze DNA extracted with **DNA-Sorb-A** (REF K-1-1/A) from:

- *Cervical swabs:*

♉ Remove excess mucus from the cervical os and surrounding ectocervix using a cotton or polyester swab. Discard this swab.

♉ Insert the Sampling Cervical Brush 1.0-1.5 centimeters into the cervical os until the largest bristles touch the ectocervix. Do not insert brush completely into the cervical canal. Rotate brush 3 full turns in a counterclockwise direction, remove from the canal.

♉ Insert brush into the nuclease-free 2,0 ml tube with 0,5 mL of Transport medium (Sacace). Vigorously agitate brush in medium for 15-20 sec.

♉ Snap off shaft at scored line, leaving brush end inside tube.

- *Tissue* homogenized with mechanical homogenizer and dissolved in PBS sterile (recommended DNA-Sorb-C REF K-1-6/50 not included in this kit, but can be ordered separately)
- *Liquid-based cytology samples* (Cytoscreen, PreservCyt) (recommended DNA-Sorb-D REF K-1-8/100 not included in this kit, but can be ordered separately)

It is recommended to process samples immediately after collection. Store samples at 2–8 °C for no longer than 24 hours, or freeze at –20/80°C. Transportation of clinical specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

AMPLIFICATION

1. Prepare required quantity of tubes for samples and controls.
2. Add for each sample in the new sterile tube **5*N µl** of **PCR-mix-1**, **10*N** of **2,5 x buffer** and **0,5*N** of **TaqF Polymerase.**
3. Add **15 µl** of **Reaction Mix** into each sample tube. Add 1 drop (25 µL = of Mineral Oil).
4. Add to appropriate tube **10 µL** of **DNA** sample obtained after sample preparation.
5. Prepare Controls as follows:
   - Negative Control: add **10 µL** of **DNA-buffer** to the tube labeled *Cneg ampl*.
   - Internal Control: add **10 µL** of **Internal Control** to the tube labeled *Cint*.
   - Positive controls: add **10 µL** of **HPV C+** to the tube labeled *Cpos*;
6. Close tubes and transfer them into the thermalcycler only when temperature reaches 95°C and start the following program:

Thermocyclers with block temperature adjustment: "PTC-100"(MJ Research) BioRad, Biometra				Thermocyclers with active temperature adjustment. "PE 2400" (Perkin Elmer), Omn-E (Hibaid) and other.		
Step	t°C	Time	Cycles	t°C	Time	Cycles
1	95°C	Pause		95°C	Pause	
2	95°C	15 min	1	95°C	15 min	1
3	95°C	30 sec	42	95°C	30 sec	42
	63°C	40 sec		63°C	30 sec	
	72°C	40 sec		72°C	30 sec	
4	72°C	1 min	1	72°C	1 min	1
5	10°C	Storage		10°C	Storage	

RESULTS ANALYSIS

Analysis of results is based on the presence or absence of specific bands of amplified DNA in Agarose gel (2%). The length of specific amplified DNA fragments is:

- **HPV – 267-325 bp**
- **Internal Control – 723 bp**

RESULTS INTERPRETATION

Table 2. Results for controls

Control	Which step of test is controlled	Specific bands in the gel **267-325 bp**	Specific bands in the gel **723 bp**	Interpretation
Neg. Control	DNA isolation	No	No	Valid result
DNA-buffer	Amplification	No	No	Valid result
Internal Control	Amplification	No	**Yes**	Valid result
HPV C+	Amplification	**Yes**	No	Valid result

PERFORMANCE CHARACTERISTICS

Analytical specificity: The analytical specificity of the primers and probes was validated with negative samples. They did not generate any signal with the specific HPV primers and probes.
Analytical sensitivity: The kit HPV High Risk Screen allows to detect HPV DNA in 100% of the tests with a sensitivity of not less than 1000 copies/ml.
Target region: E1, E2 genes

TROUBLESHOOTING

1. Controls analysis do not correspond to the listed above table 2:
a. The PCR was inhibited.
b. The reagents storage conditions didn't comply with the instructions.
♉Check the storage conditions
c. The PCR conditions didn't comply with the instructions.
♉Check the PCR conditions.
2. Positive specific band with Negative Control of extraction.
a. Contamination during DNA extraction procedure. All samples results are invalid.
♉Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol.
♉Use only filter tips during the extraction procedure. Change tips between tubes.
♉Repeat the DNA extraction with the new set of reagents.
3. Positive specific band with Negative Control of PCR (DNA-buffer).
a. Contamination during PCR preparation procedure. All samples results are invalid.
♉Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol or special DNA decontamination reagents.
♉Pipette the Positive control at last.
♉Repeat the PCR preparation with the new set of reagents.
4. Absence of the Internal Control band in a clinical sample:
a. Insufficient quantity of clinical material.
b. The PCR was inhibited.
♉Make sure that you use a recommended DNA extraction method and follow to the manufacturer's instructions.
♉Re-centrifuge all the tubes before pipetting of the extracted DNA for 2 min at maximum speed (12000-16000 g) and take carefully supernatant. Don't disturb the pellet, sorbent

inhibit reaction.
c. The reagents storage conditions didn't comply with the instructions.
♉Check the storage conditions
d. The PCR conditions didn't comply with the instructions.
♉Check the PCR conditions

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